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Rapid Diagnosis of *Brugia malayi* and *Wuchereria bancrofti* Filariasis by an Acridine Orange/Microhematocrit Tube Technique

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ABSTRACT: A microhematocrit tube technique for diagnosis of human filariasis has been previously described. A system incorporating heparin, EDTA, and acridine orange into a microhematocrit tube (Quantitative Blood Count, QBC®) has been commercially developed for the quantitation of blood counts and has been used for the diagnosis of malaria. We evaluated this test for its usefulness in the diagnosis of filariasis. Upon centrifugation, the parasites were concentrated in the area of the buffy coat and could be observed through the wall of the tube. The parasites were concentrated further by a plastic float that expands the buffy coat and confines the parasites to the periphery of the tube. Acridine orange stains the DNA of the parasite, and morphologic characteristics can be examined by fluorescence microscopy. The terminal and subterminal nuclei and long cephalic space of *Brugia malayi*, as well as the short cephalic space and caudal nuclei of *Wuchereria bancrofti*, were easily recognized and differentiated from each other. Microfilariae were detected in samples diluted to a level of approximately 50/ml.

Key words: Parasitology, DIAGNOSIS, *Brugia malayi* and *Wuchereria bancrofti*, Filariasis, Acridine Orange/Microhematocrit Tube Technique, Parasites, Biology

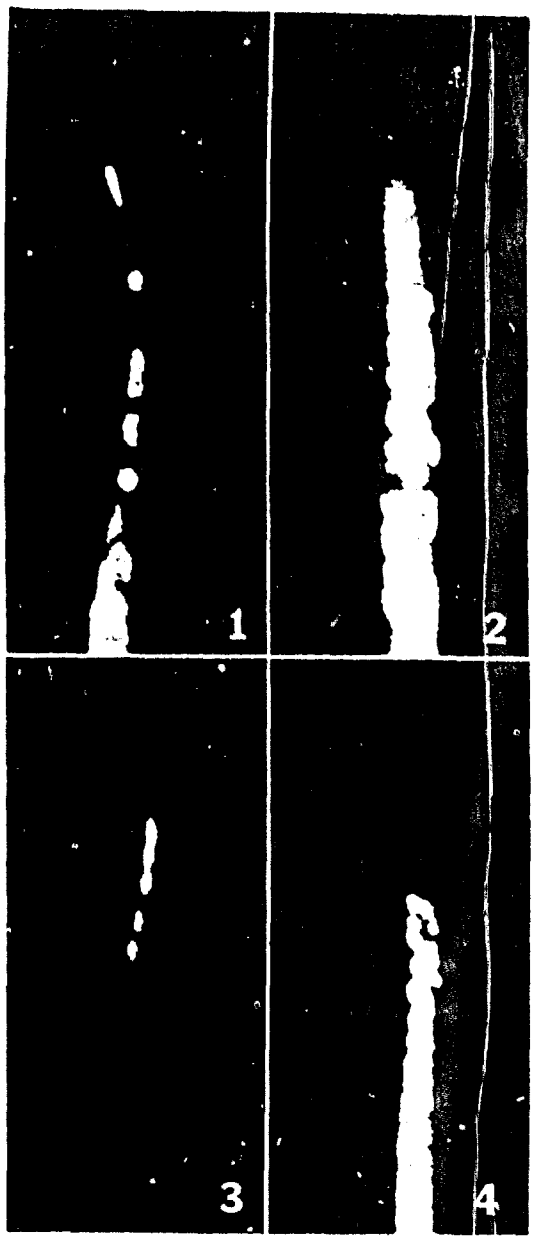
Staining of from 20 to 60 μl of blood on a thick smear is currently the most commonly used method of diagnosis of *Wuchereria bancrofti* and *Brugia malayi*. Sensitivity can be increased by filtration of from 1 to 5 ml of blood on a Nuclepore filter (Chularek and Desowitz, 1970). Both of these techniques require from 30 to 60 min. A microhematocrit tube method was described by Goldsmid (1970) and Goldsmid et al. (1972) as a rapid method for the diagnosis of human filariasis. The technique was more sensitive than a thick blood smear and can be carried out in 5–6 min.

A microhematocrit technique has been developed for the quantitation of granulocytes, lymphocytes/monocytes, and platelets and is commercially available (QBC® capillary blood tubes). These tubes have been used to diagnose *Plasmodium falciparum* malaria (Spielman et al., 1988; Rickman et al., 1989). The parasites are stained by the acridine orange dye and can be observed within the packed red blood cell layer using a 50× objective. We recently observed that these tubes could be used for the detection and identification of *B. malayi* and *W. bancrofti* microfilariae.

Brugia malayi was grown in jirds (*Meriones unguiculatus*) (Ash and Riley, 1970) and collected from the peritoneal cavity. *Wuchereria bancrofti* was collected by venipuncture from human donors in the Philippines, frozen in liquid nitrogen, and thawed immediately before use. Thawed parasites were alive and motile. Parasites were mixed with fresh heparinized human blood for study.

The QBC® tubes (Becton Dickinson, Franklin Park, New Jersey) were used according to manufacturer's instructions. The tubes were filled with blood to a premarked level (approximately 55–65 μl) and mixed with the acridine orange dye, which coats the interior of the tube. The tubes were stoppered and the plastic float was inserted. The tubes were centrifuged at 12,000 rpm for 5 min and observed by fluorescence microscopy (Olympus BH-2 with standard filter sets for fluorescein) using a 20× (SPlan 20, Olympus) objective or a 50× oil immersion (DPlan 50, Olympus) objective. The tubes were held in a trough cut in a Plexiglas block (Goldsmid et al., 1972) and covered with immersion oil to improve resolution (Woo, 1969). Thick films were prepared by spreading 10 μl of blood over an area of a microscope slide approximately 1 × 2 cm, stained with Giemsa stain, and the parasites were counted by light microscopy. In 1 experiment, 2-fold dilutions of blood were prepared and examined by both thick film and QBC®.

Both *W. bancrofti* and *B. malayi* microfilariae were observed easily using a standard 20× objective. The microfilariae were stained by acridine orange dye while in the tube. Nuclei fluoresced bright green and were clearly visible. Parasites were motile and concentrated in and around the buffy coat. Microfilariae in the plasma were clearly visible in the tube, having been displaced to the periphery by the plastic float. Microfilariae in the buffy coat were not seen as easily due to the intense fluorescence of the mononuclear and polymorphonuclear cells in this layer; however, their movement and the disturbance of the surrounding cells were detected readily. Parasites in the upper portion of the packed red cell layer could be examined easily against the dark background of the erythrocytes. The terminal and subterminal nuclei of *B. malayi* (Fig. 1) were visible, making species identification possible. The long cephalic space, also characteristic of *B. malayi*, was lightly stained but clearly identifiable (Fig. 2). The caudal nuclei (Fig. 3) and short cephalic space (Fig. 4) of *W. bancrofti* were distinct.



FIGURES 1–4. 1. Fluorescence micrograph (×500) of acridine orange-stained *Brugia malayi* in QBC® tube. The view of the posterior region shows terminal and subterminal nuclei. 2. The long cephalic space is visible on this specimen of *B. malayi* as is the lightly stained sheath. 3. This figure shows the caudal nuclei of *Wuchereria bancrofti*. 4. Anterior of *W. bancrofti*. Compare the short cephalic space of this parasite with that of *B. malayi* in Figure 2.

Microfilariae of *B. malayi* were diluted in a 2-fold series in human blood to test both sensitivity and the ability of the test to allow quantification of parasite numbers. Counts were compared to thick blood smears that were prepared and counted as described above. QBC® tubes

were prepared and examined using a 20× objective. The buffy coat was brought into focus and the parasites were counted as the tube was rotated in the Plexiglas holder. In tubes with large numbers of parasites a reference point was picked out to ensure that the entire contents of the tube were counted around the entire circumference. Counts of *B. malayi* microfilariae performed using the tubes agreed closely with the stained smears (Table I).

Microfilariae of *B. malayi* and *W. bancrofti* were detected quickly and easily in blood samples using the QBC® tubes. Due to their relative density, the microfilariae concentrated in the buffy coat region during centrifugation (Goldsmid et al., 1972). Further, the inclusion of the plastic float within the capillary tube displaced the concentrated parasites to the periphery of the tube. The distance between the float and the inner wall of the tube is approximately 40 μm, and the parasites are contained within this space. As a consequence of this and the concentration effect, almost all parasites collected in the tube were visible. Estimation of parasite counts by QBC® agreed well with direct counts of blood smears. In addition, staining of the nuclei by acridine orange made determination of species possible immediately.

In this study parasites were detected at a concentration of 50 microfilariae/ml. The theoretical limit of the test is 16 parasites/ml assuming 60 μl of blood per tube. The fact that parasites were concentrated from a relatively large volume of blood increased the sensitivity and reduced the time required for examination of the specimen. The QBC® method is less sensitive than the membrane filtration technique, which can be used to detect 1 parasite/ml. However, the QBC® has the advantage of speed and relative ease of use, and staining reagents are incorporated into the tube.

The QBC® test was found to be a simple and effective way to detect microfilariae of *W. bancrofti* and *B. malayi* quickly. The tubes are designed for blood collection by finger stick, contain EDTA (ethylenediaminetetraacetic acid) and sodium heparin, and are precoated with acridine orange. Microfilariae can be detected and counted in this system using a light microscope although species cannot be determined. Where a centralized laboratory is available specimens can be collected in the field and returned for examination. Although parasites lost motility over time, they retained their morphology over several days at room temperature (23 C) and for at least 7 days when refrigerated. We have not assessed their stability in a tropical environment or under field conditions.

TABLE I. Comparison of QBC® and thick smear techniques for quantitation of microfilariae of *Brugia malayi*.

Thick smear		QBC® tube	
Total microfilariae counted/10 μl	Parasites/ml*	Total microfilariae counted/tube	Parasites/ml†
15	1,500	‡	–
7	700	42	701
4	400	20	334
1	100	8	133
0	–	3	50

* Determined from thick film count (count times 100).
† Determined by multiplying the QBC® count by 16.7 (assuming 60 μl blood/tube).
‡ Too numerous to count.

We found the QBC® test to be a fast and simple method for detection and identification of microfilariae of *W. bancrofti* and *B. malayi*. The test can be performed from a finger stick blood collection, its sensitivity is greater than that of a thick blood smear, and it can be performed in approximately 10 min. A thick smear on the other hand requires 30 min or more for drying and staining. We believe that the test is a promising technique for the diagnosis of filariasis.

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New Host and Locality Record for *Trypanosoma peromysci*

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ABSTRACT: *Trypanosoma peromysci* Watson, 1912 (Sarcomastigophora: Kinetoplastida), is described from a new host and locality. One of 20 (5.0%) *Peromyscus leucopus* collected from Pottawatomie and Riley counties in Kansas was found to harbor the parasite. Morphometric and statistical analysis confirmed the trypanosome to be indistinguishable from *T. peromysci*, the only difference being a greater mean flagellar length than reported previously. This is the first reported occurrence of *T. peromysci* in the white-footed mouse (*Peromyscus leucopus noveboracensis* Fischer, 1829) and also the first record of its occurrence in Kansas.

Two species of trypanosome, *Trypanosoma cruzi* and *Trypanosoma peromysci*, have been reported to occur in *Peromyscus* spp. (Wood, 1934, 1942, 1952a, 1952b, 1975a, 1975b; Davis, 1952; Esquivel et al., 1967; Burkholder et al., 1980). *Trypanosoma peromysci* was first described by Watson *in* Watson and Hadwen (1912) in *Peromyscus maniculatus*, *Peromyscus nebracensis* (now *P. maniculatus nebracensis*), and other species collected from Lethbridge, Alberta, Canada. Since that time, additional hosts and localities for the parasite have been reported, including *P. maniculatus* from New Mexico, Arizona, and California (Wood, 1942, 1952a, 1975a; Davis, 1952); *Peromyscus truei* from Arizona and California (Davis, 1952; Wood, 1952a, 1975a, 1975b); *Peromyscus californicus* from California (Wood, 1942, 1952a); *Peromyscus boylii* from California (Wood, 1942, 1952a; Davis, 1952); and *Peromyscus nudipes* from Costa Rica (Esquivel et al., 1967). The parasite has not been recorded previously from *Peromyscus leucopus*.

During an ongoing survey of protozoa of small mammals in Kansas, 20 *P. leucopus* were collected from 2 locations; 17 mice were collected between 1 May and 30 June 1988 from Pottawatomie County (39°13′N, 96°30′W), and 3 mice were collected between 1 March and 30 April 1989 from Riley County (39°08′N, 96°29′W). All mice were caught using Sherman Live Traps (H. B. Sherman Traps, Tallahassee, Florida) baited with a mixture of peanut butter and oatmeal. The rodents were transported to Kansas State University where blood samples were obtained by tail bleeding into heparinized microhematocrit capillary tubes (American Scientific Products, McGaw Park, Illinois). Thick and thin blood smears were air dried and stained with either Giemsa stain or a modified Wright's stain (Leuko Stat Stain Kit, Fisher Scientific, St. Louis, Missouri). Of 20 mice, 1 (5.0%), collected from Riley County, was found to be infected. All specimens were elongate trypomastigotes with the nucleus located slightly anterior to the midpoint of the body and both the anterior and posterior ends tapering to a point (Figs. 1, 2). After morphological and statistical analysis it was concluded that the trypanosome species found was *T. peromysci*.

Davis (1952) presented a table listing measurements of 5 parameters from the specimens she examined, and Esquivel et al. (1967) listed measurements for 3 additional parameters. Because a complete description of *T. peromysci* is lacking, a description of the parasite from a new host, *P. leucopus*, is provided.

Twenty-five trypomastigotes were viewed with a Zeiss Standard RA microscope using a 100× oil immersion objective. Actual measurements and morphometric analyses were performed using the Microcom PM Interactive Image Analysis for Planar Morphometry (Southern Micro Instruments, Atlanta, Georgia) in conjunction with a Zenith ZW248-12 computer. All statistical analyses were made using the Number